

Habitat segregation of sympatric *Mnais* damselflies (Odonata: Calopterygidae): microhabitat insolation preferences and competition for territorial space

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Distribution and abundance of sympatric *Mnais costalis* and *M. pruinosa* damselflies were studied in a low mountain stream in Shiga, Japan, from 2008 through 2012. The reproductive seasons of the two species overlapped almost entirely: both species emerged in early May and disappeared in late June each year. Males of both species hold territories within the same stretch of the river; however, *M. costalis* was more abundant on the lower stream, while *M. pruinosa* was more abundant on the upper stream. Canopy openness varied at territorial sites. Results of these observations suggest that habitat segregation of the two species is due to different preference for light/shade conditions. Morisita's R_s index suggests that interspecific exclusion (or avoidance) contributes to the habitat segregation of the two species.

Keywords: Odonata; damselfly; habitat preference; light condition; exclusiveness; *Mnais costalis*; *Mnais pruinosa*

Introduction

Where two closely related species coexist in the same area, they often show microhabitat segregation and thereby avoid or reduce resource competition (e.g. Connell, 1961; Lack, 1947; Lounibos, 1981) and interspecific sexual interferences (e.g. Kaneshiro & Boake, 1987; Turner, 1994). However, it is not usually clear whether the habitat segregation is attained because the species have different preferences for environmental characteristics or because competitive interactions for space between the species generate habitat segregation.

In most of the Kinki region in central Japan, *Mnais costalis* Selys, 1869 and *M. pruinosa* Selys, 1853 coexist in the same habitats (sympatric): adults of both species emerge from the same mountain stream. Although in regions where each species exists singly (allopatric) males of both species are polymorphic (with orange-winged and clear-winged males), in our study area *M. costalis* males are monomorphically orange-winged and *M. pruinosa* males are monomorphically clear-winged (Hayashi et al., 2004; Suzuki, 1985b).

Previous field observations (Nomakuchi & Higashi, 1996; Suzuki, 1985a; Suzuki & Tamaishi, 1982) have qualitatively suggested that *M. costalis* prefers open habitat lower along a stream, while *M. pruinosa* prefers shaded forest habitat at upper stream sites. In this study, we quantified this difference using canopy openness (sky–area ratio calculated from a hemispherical photograph)

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as a measure of relative insolation at each location. We tested whether canopy openness was correlated with habitat preference of each species. We analyzed the effect of conspecific and heterospecific male density on the distribution of males.

Methods

Study site and census method

The study was carried out at a mountain stream surrounded by trees and bushes (less than 30 cm deep and 1–10 m wide), in Otsu, Shiga Prefecture, Kinki region (34°55′49″ N, 135°56′31″ E, 140 m in elevation). In four years (2008, 2010, 2011 and 2012), we conducted capture-mark-release-resighting censuses of both species along a 480 m stretch of river throughout the reproductive season. To facilitate location of each individual found, we placed a numbered tag every 10 m along the stream (47 sites). Unmarked individuals were captured with an insect net, the site number recorded, their left hind wing and abdominal lengths measured, and the animal given an individual number on the hind wing with a quick-drying color marker and then released.

In 2008, 2010, 2011 and 2012, we performed censuses on 20 days from 8 May to 16 June, 16 days from 15 May to 5 June, 15 days from 16 May to 25 June, and 39 days from 1 May to 18 June, respectively.

Measurement of canopy openness

As a measure of insolation conditions, we measured the canopy openness of potential territorial sites that were occupied at least once by one male during the study period. We took hemispherical photographs using a Nikon fisheye converter fixed at 0.3 m above the water surface at the center of each territorial site in early June (around peak reproductive season). We calculated canopy openness using free software canopOn2 (<http://takenaka-akio.org/etc/canopon2/index.html>).

Spatial overlap of two species

To determine whether the two species use the sites along the stream exclusively or concomitantly, we used Morisita's index of overlap, R_δ (Morisita, 1959). The index of correlation, R_δ , between *M. costalis* (c) and *M. pruinosa* (p) is described as:

$$R_\delta = C_\delta - W_\delta \quad (R_\delta \geq 0) \quad (1)$$

or

$$R_\delta = (C_\delta - W_\delta) / W_\delta \quad (R_\delta < 0), \quad (2)$$

where $W_\delta = 2/(\delta_c + \delta_p)q$, q is the number of observation sites. δ for each species and C_δ is defined as:

$$\delta_c = \sum_{i=1}^q n_{ci}(n_{ci} - 1) / N_c(N_c - 1) \quad (3)$$

$$\delta_p = \sum_{i=1}^q n_{pi}(n_{pi} - 1) / N_p(N_p - 1) \quad (4)$$

and

$$C_{\delta} = \sum_{i=1}^q n_{ci}n_{pi}/(\delta_c + \delta_p)N_cN_p, \quad (5)$$

where n_{ci} and n_{pi} are the numbers of observations at the i th site for each species and N_c and N_p are the total numbers of observations for each species. R_{δ} takes a value of zero if two species occupy each site independently of one another. When no site is utilized by both species (complete exclusiveness), R_{δ} becomes -1 , and when occupied sites are always used by both species (complete co-occurrence), it becomes $+1$. We calculated R_{δ} separately for each year to examine if population density of each species had any influence on R_{δ} .

Results

Body sizes

Table 1 shows characteristics of *M. costalis* and *M. pruinosa*. We measured left hind wing and abdominal lengths of individuals every year. The left hind wing and abdominal lengths of *M. costalis* were significantly larger than those of *M. pruinosa* in both sexes. It was shown that the body size fluctuates annually, but the relative difference in body size between species or between sexes was not changed (Table 2).

Annual fluctuation of abundances

Table 3 shows the number of damselflies marked and the total number of individuals sighted in each year, with males (a) and females (b) shown separately. As the census intensity was different among study years, the abundances of males and females of the two species in each year were represented as the total number of individuals divided by the number of census days. The abundances fluctuated between years, but the species changes were almost synchronous: both species were abundant in

Table 1. Left hind wing and abdominal lengths of *M. costalis* and *M. pruinosa* in four study years.

Year	Sex	Character	<i>M. costalis</i>		<i>M. pruinosa</i>	
			Mean length (mm) \pm S.D.	<i>N</i>	Mean length (mm) \pm S.D.	<i>N</i>
2008	Male	Left hind wing	37.678 \pm 1.791	118	33.201 \pm 1.319	162
		Abdomen	45.301 \pm 2.183	118	41.177 \pm 1.807	162
	Female	Left hind wing	37.560 \pm 1.653	50	34.710 \pm 1.551	62
		Abdomen	40.300 \pm 2.063	50	37.596 \pm 1.708	62
2010	Male	Left hind wing	37.845 \pm 1.647	55	33.104 \pm 1.425	144
		Abdomen	45.145 \pm 2.076	55	40.344 \pm 1.947	147
	Female	Left hind wing	36.989 \pm 1.579	46	34.494 \pm 1.672	85
		Abdomen	38.641 \pm 2.635	46	36.594 \pm 1.434	85
2011	Male	Left hind wing	37.913 \pm 1.145	103	33.519 \pm 1.661	135
		Abdomen	45.316 \pm 1.721	103	40.926 \pm 2.380	135
	Female	Left hind wing	37.595 \pm 1.258	58	34.636 \pm 1.829	55
		Abdomen	40.207 \pm 1.691	58	36.836 \pm 1.826	55
2012	Male	Left hind wing	37.810 \pm 1.421	509	33.489 \pm 1.580	368
		Abdomen	45.869 \pm 1.916	512	41.366 \pm 1.816	372
	Female	Left hind wing	37.409 \pm 1.619	370	34.539 \pm 1.510	163
		Abdomen	40.228 \pm 1.667	371	37.672 \pm 2.116	163

Table 2. Two-way ANOVAs of left hind wings and abdomens for both sexes in relation to species and year.

Independent variables	Male				Female			
	Left hind wing		Abdomen		Left hind wing		Abdomen	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Species	3579.857	<0.001	2215.726	<0.001	1730.610	<0.001	1709.610	<0.001
Year	2.968	0.031	15.099	<0.001	1.209	0.305	54.52	<0.001
Species × year	0.974	0.404	1.205	0.306	0.521	0.668	8.73	0.051

Table 3. Number of males (a) and females (b) marked and total individuals (damselfly–days) and the average abundance in each year.

(a)	<i>Mnais costalis</i> male			<i>Mnais pruinosa</i> male			Morishita's <i>R</i> _δ
	No. males marked	Total males sighted	No. males/day/10 m	No. males marked	Total males sighted	No. males/day/10 m	
Year							
2008	193	826	0.860	250	962	1.002	−0.405
2010	55	353	0.460	149	523	0.681	−0.678
2011	107	291	0.404	131	302	0.419	−0.540
2012	514	2175	1.162	373	1396	0.746	−0.464
(b)	<i>Mnais costalis</i> female			<i>Mnais pruinosa</i> female			No. females/day/10 m
	No. females marked	Total females sighted	No. females/day/10 m	No. females marked	Total females sighted	No. females/day/10 m	
Year							
2008	112	202	0.210	109	167	0.174	
2010	46	103	0.134	81	138	0.180	
2011	60	85	0.118	53	67	0.093	
2012	372	758	0.405	163	251	0.134	

2008 and 2012, while they were less abundant in 2010 and 2011 (Table 3). Only male data are used for later analyses because females are much less likely to be found than males.

Seasonal overlap of reproductive periods

Figure 1 shows the seasonal changes in the number of *M. costalis* and *M. pruinosa* males. The start of a new reproductive season was variable, probably due to weather conditions in late April and early May. Every year, the two species started their reproductive seasons almost synchronously. The termination of the reproductive season was also variable among years, but both species disappeared almost at the same time every year. It appears that in 2011 they started the reproductive season late and also finished it later than in other years (Figure 1c). Despite annual fluctuations, the reproductive periods of the two species overlapped almost entirely in all four years.

Spatial overlap of two species

Figure 2 shows the abundances of *M. costalis* and *M. pruinosa* males along the study stream. Both species are recorded from almost all sites of the study stream every year; however the relative abundance changed from site to site. Every year, *M. costalis* males were abundant around the 20–50 m and 190–240 m sites, while *M. pruinosa* males were abundant around the 400–450 m sites. In 2008 and 2012 when the density of *M. costalis* was relatively high, however, they were abundant also around the 350–450 m sites.

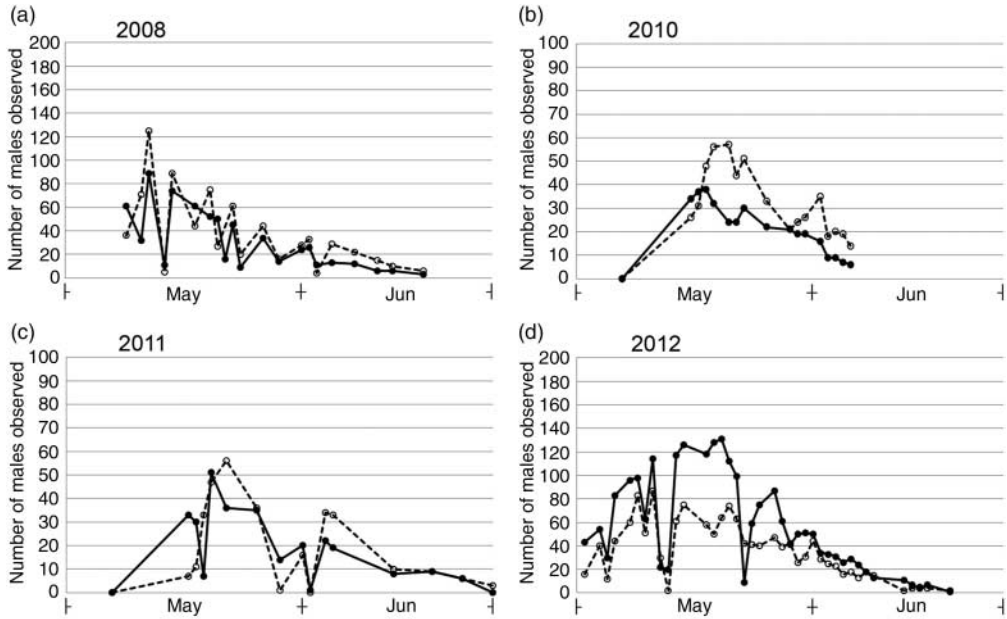


Figure 1. Seasonal changes in the number of *M. costalis* and *M. pruinosa* males observed in (a) 2008, (b) 2010, (c) 2011 and (d) 2012. Solid and open circles indicate *M. costalis* and *M. pruinosa*, respectively.

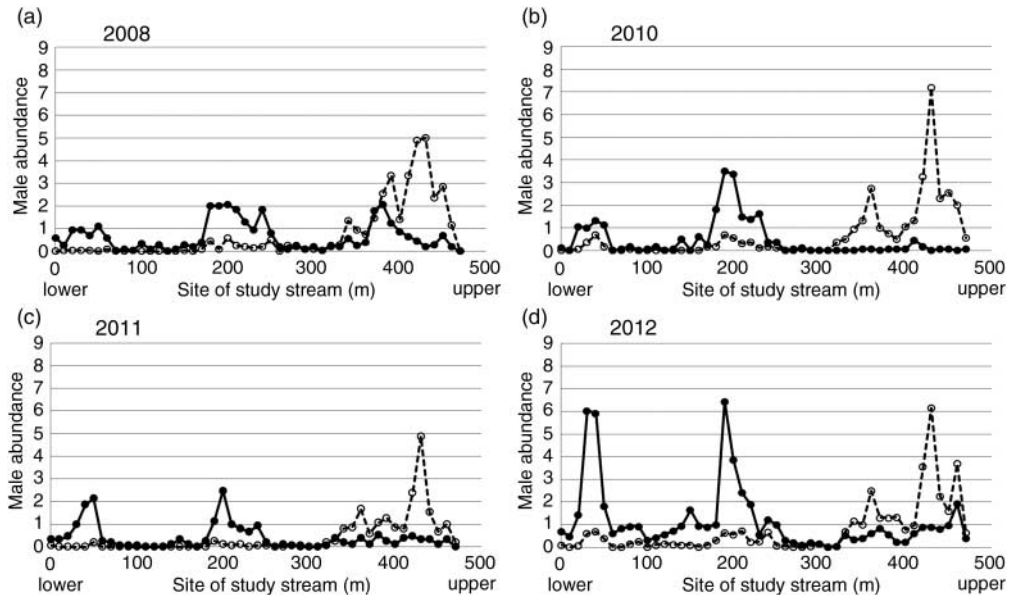


Figure 2. Abundance of *M. costalis* and *M. pruinosa* males along the study stream in (a) 2008, (b) 2010, (c) 2011 and (d) 2012. Abundance is represented by the average number of males observed at each site through a reproductive season. Solid and open circles indicate *M. costalis* and *M. pruinosa*, respectively.

Morisita's R_s index was consistently smaller than zero every year (Table 3), which suggested that the two species distribute themselves exclusively rather than distributing independently. There was no significant effect of male density of either species on the R_s value (ANOVA, $F = 0.561$, $p = 0.702$).

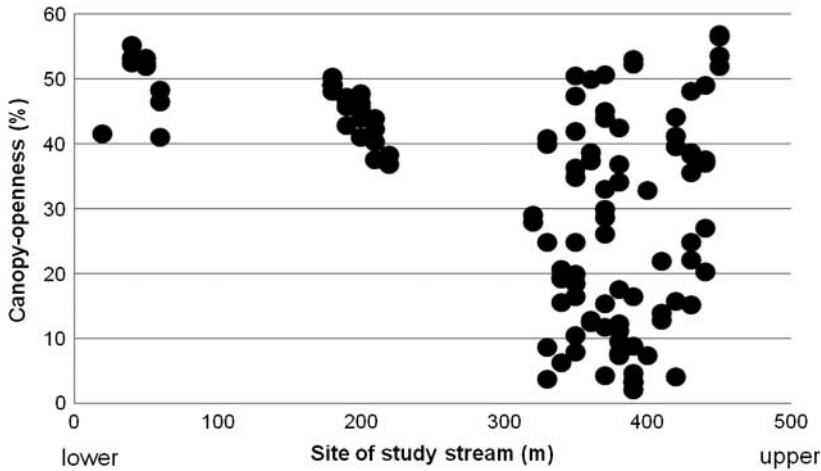


Figure 3. Canopy openness at males' territorial sites in relation to position along the stream.

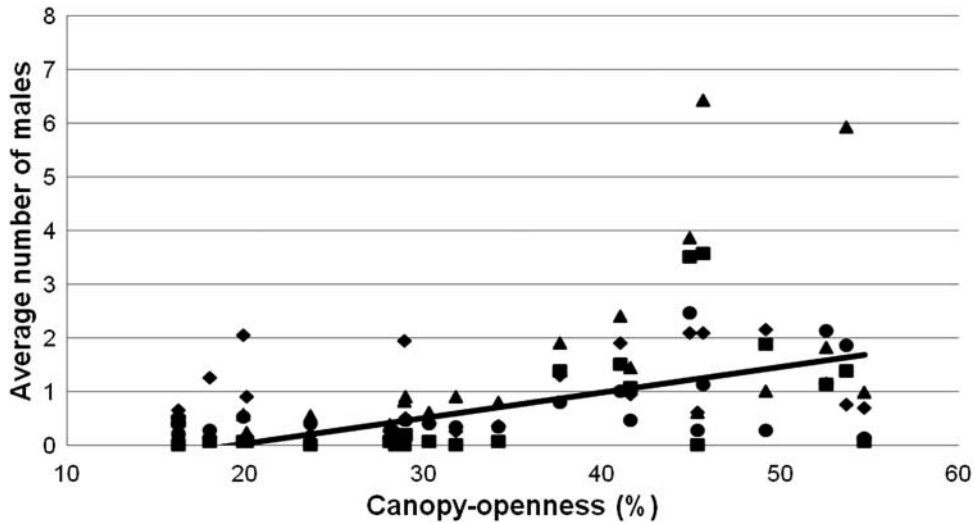


Figure 4. Relationship between the abundance of *M. costalis* males and the canopy openness at each site. Solid line is the regression line for all years combined. ♦: 2008, ■: 2010, ●: 2011, ▲: 2012.

Canopy openness and male abundance

Canopy openness of potential territorial sites was generally high (40–60%) at lower parts of the study stream, while it was highly variable (0–60%) at upper parts of stream (Figure 3). In *M. costalis*, the male abundance (average number of males sighted at each site) increased with the increase in canopy openness. By contrast, in *M. pruinosa* it decreased with the increase in canopy openness (Figures 4, 5; Table 4).

Discussion

We described seasonal population changes of *M. costalis* and *M. pruinosa* on a low elevation mountain stream in Shiga prefecture, Japan, where these two species coexist. From four-year

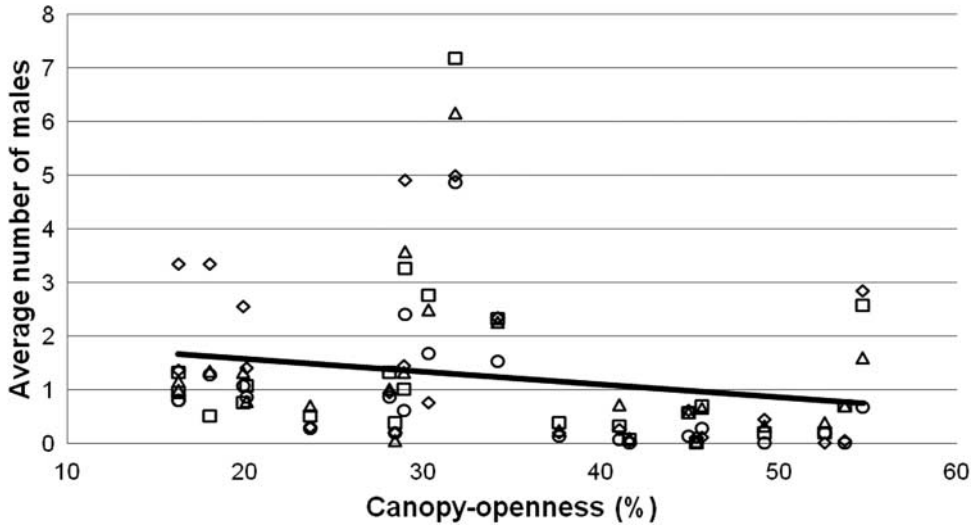


Figure 5. Relationship between the abundance of *M. pruinosa* males and the canopy openness at each sites. Solid line is the regression line for all years combined. \diamond : 2008, \square : 2010, \circ : 2011, Δ : 2012.

Table 4. Regression analysis of the average number of observed individuals (y) as a function of the canopy openness at each site (x) for *M. costalis* and *M. pruinosa*.

	Regression equation	r	p
<i>Mnais costalis</i>	$y = 0.043x - 0.541$	0.462	<0.001
<i>Mnais pruinosa</i>	$y = -0.031x + 2.234$	0.267	0.010

censuses, we found that initiation and termination of the reproductive seasons of the two species overlap almost entirely despite their annual fluctuations. The relative abundance of the species changed from the lower to the upper stream suggesting a habitat segregation of the two species: *Mnais costalis* was abundant on the lower stream, and *M. pruinosa* was abundant on the upper stream. Though this pattern has been reported in several *Mnais* studies (Nomakuchi & Higashi, 1996; Suzuki, 1985a), we have directly confirmed the stability of these spatial relationships based on long term studies.

We also found that some sites are consistently preferred by *M. costalis*, and other sites are consistently preferred by *M. pruinosa*. The overlap in distributions became larger when the population densities of both species were high, but there was no significant increase in Morisita's R_s index with the increase of male density of either species. Together these results suggest that *M. costalis* prefers environments on the lower stream with little influence from *M. pruinosa*, while *M. pruinosa* prefers environments on the upper stream almost independently of *M. costalis*.

Habitat segregation of sympatric related species is often explained as a result of resource partitioning (e.g. Connell, 1961; Lack, 1947; Lounibos, 1981). However, this is not a likely explanation in *Mnais* damselflies because they use forest streams almost exclusively for mating and oviposition. Immature adults of both species spend about a week feeding on small insects in the forest canopy, where both species mix and are apparently not agonistic toward each other. Even after maturation, both males and females spend early morning and late afternoon feeding in the tree canopy about 10 m away from the water area. Therefore, habitat segregation in *Mnais* should be regarded to be an adaptation to reduce interspecific reproductive interference (Gröning & Hochkirch, 2008).

We suggest that one of key environmental features affecting habitat segregation of *M. costalis* and *M. pruinosa* is the heterogeneous insolation within a forest. Forests have a complex mosaic of

light environments that differ in color and brightness (Endler, 1993) as well as a mosaic of thermal environments that differ between shade and light (Tsubaki et al., 2010). Measurement of canopy openness revealed that *M. costalis* preferred sunny habitats while *M. pruinosa* preferred shady habitats in this study. Figures 4 and 5 clearly show the difference in habitat preference between *M. costalis* and *M. pruinosa*; it appears that *M. costalis* and *M. pruinosa* show preferences for around 50% and 30% canopy openness, respectively. This means that both species prefer habitats with small gaps and forest shade, but *M. costalis* prefers slightly larger gaps than *M. pruinosa*. Very large gaps or completely shaded sites are not preferred by either species.

The advantages and disadvantages of selecting sunny habitat (or shady habitat) have been discussed mainly in the context of crypsis and conspicuousness. For example, guppies (*Poecilia reticulata*, Poeciliidae) are small fish that live in clear mountain streams running through tropical rain forests of northeastern South America. Guppies are genetically polymorphic for male color patterns, and the color patterns of a particular local population present a balance between predation and sexual selection (Endler, 1983, 1995). Although bird predation is biased toward orange-winged *M. costalis* males (Tsubaki, unpublished data), predation is not a likely explanation for the habitat segregation of *Mnais* damselflies: conspicuous *M. costalis* males prefer sunny habitat and relatively cryptic *M. pruinosa* males prefer shady habitat.

Species-specific differences in physiological responses to abiotic factors of habitats may lead to habitat segregation (Huey, 1991). For ectotherms, habitat thermal conditions are an important factor for habitat selection, because thermal conditions strongly influence the degree to which ectotherms can perform physiological and behavioral activities (Huey, 1991), and this will therefore influence fitness (Huey & Berrigan, 2001; Tsubaki et al., 2010; Willmer, 1991).

Tsubaki et al. (2010) studied copulation success of males in relation to insolation conditions of territorial sites and showed that *M. costalis* males were successful at sunny territorial sites. This was partly because males at sunny sites have higher body temperatures and are able to perform intensive courtship displays toward females. In contrast, mating success of *M. pruinosa* males was not influenced by the insolation condition at territorial sites, probably because males of this species do not show any courtship display (Tsubaki, unpublished data).

The difference in body size between *M. costalis* and *M. pruinosa* males may partly explain why *M. costalis* prefers sunny habitat while *M. pruinosa* prefers shady habitat. Samejima and Tsubaki (2010) showed that flight performance of *M. costalis* males is influenced by body temperature and body size. Moreover, minimum body temperature for flight is known to be lower in smaller dragonflies (May, 1976), and in smaller individuals in *Mnais* damselflies (Samejima, unpublished data).

Although physiological responses to the abiotic environment seem to be the most likely explanation of habitat segregation of *Mnais* damselflies, we do not dismiss the influence of interspecific interactions. We have studied the sympatric/allopatric distribution of the *Mnais* damselflies for more than 15 years. In allopatric regions, both *M. costalis* and *M. pruinosa* show male color polymorphism, and both species prefer relatively shady habitat (Okuyama, unpublished). Therefore, habitat segregation in response to a heterogeneous light environment seems to be a consequence or, conversely, a cause of character displacement in multiple traits associated with habitat preference (i.e. body size, wing color and flight ability). Such evolutionary character shifts may have already been completed in sympatric regions. Studies on geographic variation of character divergence in *Mnais* damselflies are now ongoing, and the results will be reported elsewhere (Okuyama, unpublished).

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